Final Report for "Using a Combined QM/MM Approach with CPMD-Rare Event Methods to Study Enzymatic Reaction Mechanisms"

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1. Executive Summary

Despite decades of study, the mechanism by which orotidine-5'-monophosphate decarboxylase (ODCase) catalyzes the decarboxylation of orotidine monophosphate remains unresolved. A computational investigation of the direct decarboxylation mechanism has been performed using mixed quantum mechanical/molecular mechanical (QM/MM) dynamics simulations. The study was performed with the program CP2K that integrates classical dynamics and *ab initio* dynamics based on the Born-Oppenheimer approach. Two different QM regions were explored. The free energy barriers for direct decarboxylation of orotidine-5'-monophosphate (OMP) in solution and in the enzyme (using the larger QM region) were determined with the metadynamics method to be 40 kcal/mol and 33 kcal/mol, respectively. The calculated change in activation free energy ($\Delta\Delta G^{\ddagger}$) on going from solution to the enzyme is therefore -7 kcal/mol, far less than the experimental change of -23 kcal/mol.[1] These results do not support the direct decarboxylation mechanism that has been proposed for the enzyme. However, in the context of QM/MM calculations, it was found that the size of the QM region has a dramatic effect on the calculated reaction barrier.

2. Project Motivation

Orotidine-5'-monophosphate decarboxylase (ODCase) was declared the most proficient enzyme known over a decade ago.[1] Since then, there have been many experimental and computational studies attempting to elucidate the mechanism by which this enzyme accelerates the rate of spontaneous decarboxylation of orotidine-5'-monphosphate (OMP) in solution by more than 17 orders of magnitude (Figure 1, a). Surprisingly, the enzyme does not use metal ions or other cofactors, which are commonly essential in other decarboxylases.[2,3,4] The direct decarboxylation mechanism has gained support in recent years. The direct decarboxylation mechanism for OMP involves stretching of the C6-CO_2 bond, and leads to formation of carbon dioxide and a deprotonated uridine with an unstabilized carbanion at C6 (Figure 1, b). We set out to use the most sophisticated QM/MM technique that has been applied to the system to date, to calculate the free energy barrier for direct decarboxylation in ODCase.

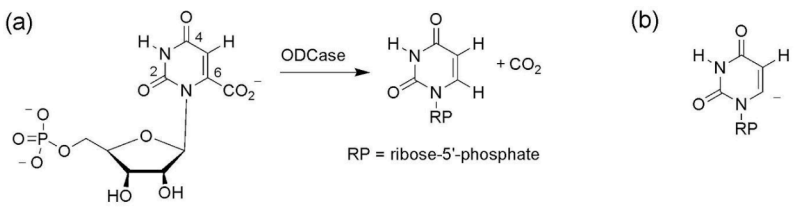

Figure 1. (a) The decarboxylation of OMP to UMP. (b) The carbanion intermediate formed by direct decarboxylation of OMP.

3. Technical Approach

Available computer power does not typically allow for adequate sampling in molecular dynamics simulations of large molecules to observe rare events like chemical reactions. Metadynamics is a nonequilibrium method that allows for the system to escape minima in order to sample the rest of the free energy surface on a timescale that is accessible by present day computers.[5,6] The metadynamics method has been used in a number of applications, including the investigation of bacterial chloride channels,[7] deprotonation of formic acid,[8] and flexible ligand docking.[9] The method is based on the assumption that it is possible to define a set of collective coordinates that can distinguish between reactants and products, and can sample the low-energy reaction paths. Collective variables (CVs) must be functions of the ionic coordinates; examples include bond lengths, dihedral angles, coordination numbers, etc. A history-dependent repulsive potential is built up in low-energy wells by adding a biasing potential term along the CVs at each metadynamic step in the form of a small Gaussian "hill" (equation 1). As the hills build up along the CVs, the system is forced to escape local minima and to explore higher energy regions of the free energy surface (FES). In the limit of infinite time, the biasing potential exactly cancels the underlying FES along the CVs (equation 2):

$$V_{bias}(\mathbf{s},t) = \sum_{t_i} H \exp\left(\frac{\left|\mathbf{s} - s(t_i)\right|^2}{2\omega^2} \right) \qquad (1)$$

$$F(\mathbf{s}) = -V_{bias}(\mathbf{s},t)_{t\to\infty} \qquad (2)$$

V_{bias} is the repulsive biasing potential term, which is a function of the CVs, **s,** and time, t, with hill parameters having height H and width ω. The FES, $F(\mathbf{s})$, can be reconstructed along the CVs given a sufficient amount of time.

Metadynamics was implemented into the *ab initio* molecular dynamics program CP2K, which was developed in part by I.-Feng W. Kuo and Christopher J. Mundy at the Lawrence Livermore National Laboratory.[10] All QM/MM calculations were performed using the software suite CP2K, and run using the computer resources at LLNL, on several computer clusters, including thunder, zeus, and atlas.

4. Research or Other Technical Results

The direct decarboxylation mechanism of OMP has been investigated by a mixed QM/MM *ab initio* MD study on the entire enzyme/substrate system and in solution. The enzymatic barrier was calculated to be 16 kcal/mol higher than the experimental barrier, while the barrier in solution only deviated by 1 kcal/mol as compared to experiment. The results from the QM/MM study do not support the direct decarboxylation mechanism as the reaction catalyzed by ODCase. However, it was also found that the choice of QM region can have a significant effect on the predicted reaction barrier. A small QM region does not appear to be sufficient to accurately model this reaction in the enzyme, and increasing the size of the QM region tends to increase the calculated barrier.

Explorations of other mechanisms for the decarboxylation of OMP in ODCase with this QM/MM methodology are in progress. Alternative mechanisms that are currently being investigated include those involveing Schiff-base formation at C4, concerted O4-protonation/decarboxylation, concerted C5-protonation/decarboxylation, and Michael addition at C5.

5. Papers and Book Chapters Supported in Part by the Subcontract

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6. References

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[10] See http://cp2k.berlios.de/ for more information on CP2K